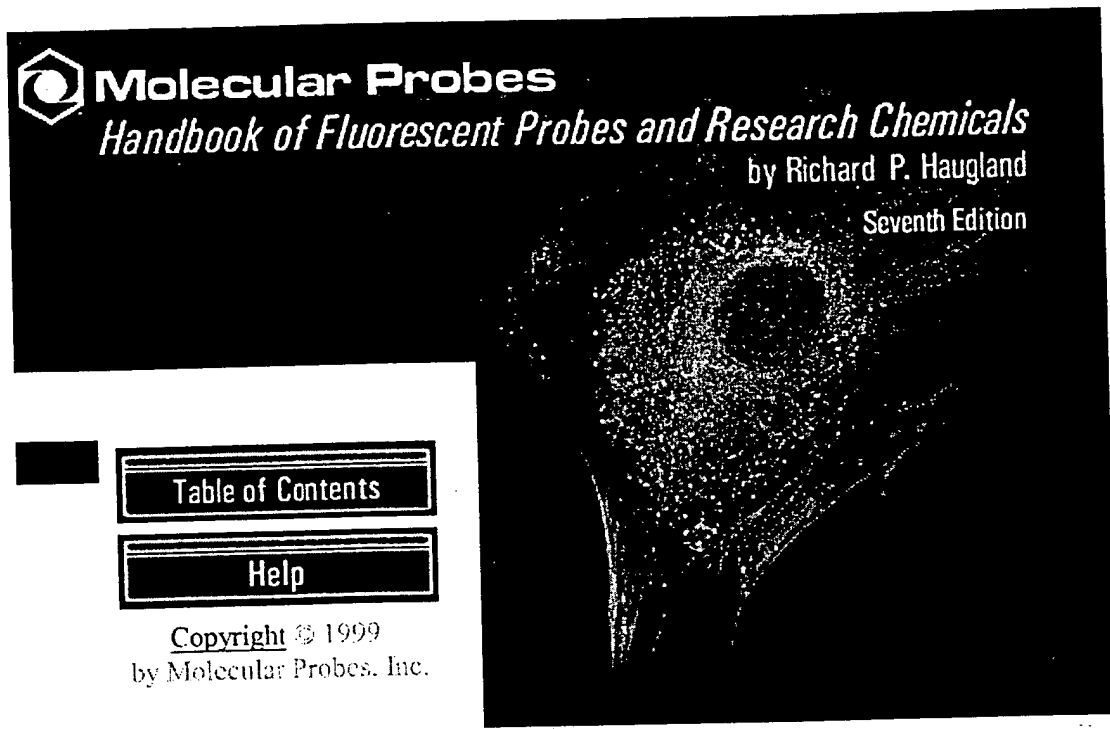


EXIBIT 2



Molecular Probes
Handbook of Fluorescent Probes and Research Chemicals
by Richard P. Haugland
Seventh Edition

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Handbook of Fluorescent Probes and Research Chemicals

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A Letter from the President

Dear Researcher:

The Seventh Edition of Molecular Probes' *Handbook of Fluorescent Probes and Research Chemicals* completely updates and expands the Sixth Edition of the *Handbook*, which was published in November of 1996. This CD-ROM edition duplicates much of the information that is available at our popular Web site, including the full text of the *Handbook*, a bibliography with over 32,000 citations linked to our products, chemical structures, full spectra for many compounds, a gallery of over 200 images, product information sheets for many products and much more. Molecular Probes has developed several particularly important new products since the publication of the Sixth Edition of the *Handbook*, including our Alexa Fluor dyes, SYBR Gold nucleic acid stain and our ultrasensitive SYPRO Ruby protein stains — these are fully described in this edition. The text has been completely revised and, in some cases, chapters or sections have been reorganized or combined to make it easier for you to locate product information. In addition, the CD-ROM is completely searchable.

The medium of the CD-ROM is ideal for communicating the wealth of information in the databases that we maintain on our products and their applications. Because the content of this CD-ROM is fixed until we publish another edition, we invite you to visit our Web site frequently for updates on new and existing products. To receive some of this information directly, Subscribe to Our E-mail Newsletter. We are already working on the next print edition of the *Handbook*, with an accompanying CD-ROM. We always welcome your comments and suggestions for improving the *Handbook* and our Web site, or for new products that you feel would be useful for your research.

Sincerely,

Richard P. Haugland

Richard P. Haugland, Ph.D.
President
Molecular Probes, Inc.

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Chapter 20 — Indicators for Ca²⁺, Mg²⁺, Zn²⁺ and Other Metals

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20.1 Introduction to Ca²⁺ Measurements with Fluorescent Indicators

Fluorescent probes that show a spectral response upon binding Ca²⁺ have enabled researchers to investigate changes in intracellular free Ca²⁺ concentrations using fluorescence microscopy, flow cytometry and fluorescence spectroscopy. ^{REF} These fluorescent indicators, most of which are derivatives of the Ca²⁺ chelators EGTA, APTRA and BAPTA, ^{REF} have evolved largely through the efforts of Roger Tsien and his colleagues and, more recently, through those of scientists at Molecular Probes.

Selection Criteria for Fluorescent Ca²⁺ Indicators

Molecular Probes offers the widest available selection of fluorescent Ca²⁺ indicators for detecting changes in intracellular Ca²⁺ over the range of <50 nM to >50 μM (Table 20.1). Not only are we the primary supplier of fura-2, indo-1, fluo-3 and rhod-2, but we exclusively offer a number of other indicators for intracellular Ca²⁺. Our new fura-4F, fura-5F and fura-6F indicators provide increased response sensitivity to intracellular Ca²⁺ concentration in the 0.5–5 μM range compared to fura-2. Our fluo-4, fluo-5F, fluo-5N, Oregon Green 488 BAPTA, Calcium Green, X-rhod-1 and Fura Red indicators allow Ca²⁺ detection over a wide concentration range and offer increased brightness and reduced phototoxicity. We also offer indicators that are conjugated to high- or low-molecular weight dextrans for improved cellular retention and less compartmentalization, as well as lipophilic Ca²⁺ indicators for possible use in studying near-membrane Ca²⁺ (Section 20.4). Molecular Probes strives to provide the highest-purity indicators available anywhere. The AM ester forms of most of our indicators are typically at least 95% pure by HPLC analysis, although purity often exceeds 98%. Furthermore, the AM esters of many of the Ca²⁺ and Mg²⁺ indicators are available in special packaging for more convenient handling and for reduced risk of deterioration during storage.

A number of factors should be considered when selecting a fluorescent Ca²⁺ indicator, some of which are summarized in Table 20.1 and include the:

- **Indicator form** (salt, AM ester or dextran), which influences the cell-loading method and affects the indicator's intracellular distribution and retention. The salt and dextran forms are typically loaded by microinjection, electroporation, patch-pipette perfusion or by using our Influx pinocytic cell-loading reagent (I-14400, I-14402, Section 20.8). In contrast, the cell-permeant acetoxymethyl (AM) esters can be passively loaded into cells, where they are cleaved to cell-impermeant products by intracellular esterases. For a discussion of ratiometric methods and AM ester loading, see Loading and Calibration of Intracellular Ion Indicators.
- **Measurement mode**, which is dictated by whether qualitative or quantitative ion concentration data, is required. Ion indicators that exhibit spectral shifts upon ion binding can be used for

rationometric measurements of Ca²⁺ concentration, which are essentially independent of uneven dye loading, cell thickness, photobleaching and dye leakage. Excitation and emission wavelength preferences depend on the type of instrumentation being used, as well as on sample autofluorescence and on the presence of other fluorescent or photoactivatable probes in the experiment.

- **Dissociation constant (K_d)**, which must be compatible with the Ca²⁺ concentration range of interest. Indicators have a detectable response in the concentration range from approximately 0.1 × K_d to 10 × K_d. For ratiometric indicators, the Ca²⁺ response range is also somewhat dependent on the measurement wavelengths used. ^{REF} The K_d of Ca²⁺ indicators is dependent on many factors, including pH, temperature, ionic strength, viscosity, protein binding and the presence of Mg²⁺ and other ions. Consequently, K_d values for intracellular indicators are usually significantly higher than corresponding values measured in cell-free solutions (Table 20.8).

Intracellular calibration of Ca²⁺ indicators may be achieved either by manipulating Ca²⁺ levels inside cells using an ionophore or by releasing the indicator into the surrounding medium of known Ca²⁺ concentration via detergent lysis of the cells. We offer several control compounds and buffers for measuring and manipulating intracellular and extracellular Ca²⁺, which are discussed in Section 20.8. These include caged-Ca²⁺ reagents and caged chelators (NP-EGTA, DMNP-EDTA and diazo-2), as well as Calcium Calibration Buffer Kits, BAPTA-derived buffers, ion-selective chelating polymers (Calcium Sponge products) and the Ca²⁺ ionophores A-23187 and its nonfluorescent analog, 4-bromo A-23187. Our reagents for probing Ca²⁺ regulation and second messenger activity are described in more detail in Chapter 18.

Reference Guides for Using Fluorescent Ca²⁺ Indicators

In order to meet the needs of researchers new to this technology, Molecular Probes offers selected books that provide surveys of fluorescent ion indicators and techniques for using them.

- *Methods in Cell Biology, Volume 40: A Practical Guide to the Study of Calcium in Living Cells* (M-7890), edited by Nuccitelli, is an indispensable guide for all researchers using fluorescent ion indicators.
- *Calcium Signaling Protocols (Methods in Molecular Biology, Volume 114)* (C-14945), edited by Lambert, provides optimized protocols for routine fluorometric Ca²⁺ measurements, as well as for confocal microscopy, subcellular Ca²⁺ imaging, Ca²⁺ channel activity determinations and detection of Ca²⁺ release from intracellular stores.
- *Fluorescent and Luminescent Probes for Biological Activity: A Practical Guide to Technology for Quantitative Real-Time Analysis, Second Edition* (F-14944), edited by Mason, is a comprehensive survey of optical probe techniques, including fluorescent ion indicators.

Other reviews of these indicators include those by Silver, ^{REF} Scheenen *et al.*, ^{REF} and Kao. ^{REF} Several earlier reviews on ion indicators also contain useful technical information. ^{REF}



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